

# Isomerisation of lactose to lactulose using milk concentration permeate and oyster shell powder

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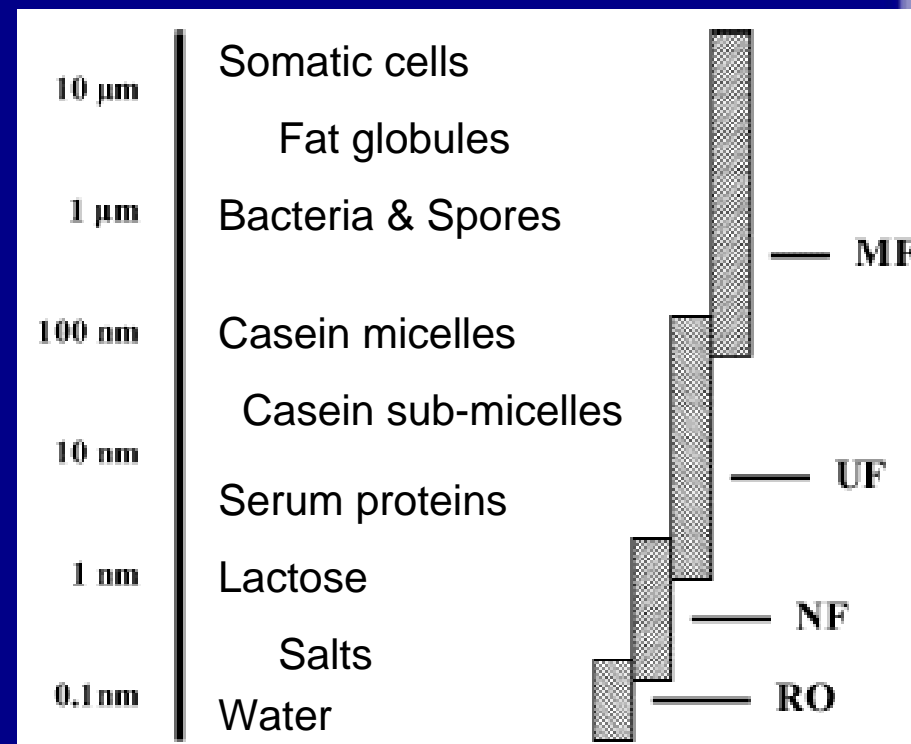


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# Milk Concentration Permeate (MCP)

- ❑ Australian dairy plants produce high volumes of MCP from the increased utilisation of ultrafiltration (UF) for pre-concentration of milk
- ❑ M<sub>cp</sub> is a low-value by-product not suitable for human consumption
- ❑ M<sub>cp</sub> has a high BOD and needs proper treatment before disposal



**Figure 1. Component in milk: Size indication and membrane processes**

## Comparative composition of MCP (g/L)

Products	Protein	Lactose	Fat	Ash	Ca
Skim milk	33	48	15	7	1.2
Whey	8.5	48.5	0.75	9.2	4.6
MCP	4.2	48	1	8.5	3.3

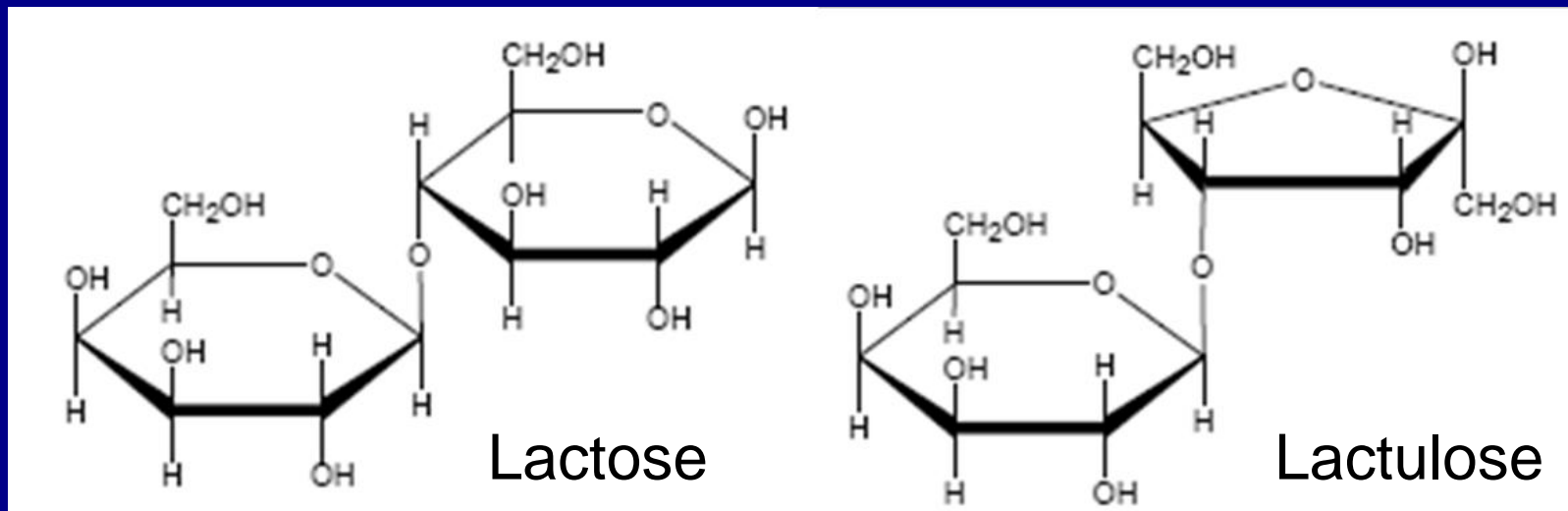
**Source:** Atra et al., 2005

# Utilisation opportunities for MCP

- ❑ Feeding farm animals
- ❑ Blending with other dairy liquids
- ❑ Recovering lactose for use in food formulation
- ❑ Using as a cheap lactose source to produce lactose-derivatives e.g. lactulose, lactitol and lactobionic acid

# Lactulose

- ❑ A synthetic ketose disaccharide (4-O- $\beta$ -D-galactopyranosyl-D-fructose)
- ❑ Neither metabolised nor absorbed in small intestine
- ❑ Bacterially fermented in the colon to short chain fatty acids and gases.



**Figure 2.** Basic structure of lactose and lactulose

# Lactulose

- ❑ Used for treatment of several intestinal disorders
- ❑ Used as a prebiotic in infant formulas and health foods
- ❑ Formed in heated dairy products depending on the severity of heat treatment
- ❑ Generated via the Lobry de Bruyn-Alberda van Ekenstein isomerisation of lactose under alkaline condition (Andrews, 1989)

# Previous isomerisation studies

## Catalysts

□ Strong organic alkaline reagents e.g. tertiary amines

□ Strong alkaline reagents e.g.  $\text{Ca(OH)}_2$ , NaOH, KOH

□ Complexing reagents e.g. borate and aluminate

## Researchers

Parrish, 1970

Montgomery & Hudson, 1930

Nagasawa *et al.*, 1974

Deya & Takahashi, 1991

Dendene *et al.*, 1994

Zokae *et al.*, 2002

Hicks & Parrish, 1980

Krumbholz & Dorscheid, 1991

Kozempel & Kurantz, 1994

Mahran *et al.*, 1995

Carobbi *et al.*, 2001

Zokae *et al.*, 2002

# Catalysts employed for isomerisation

## Catalysts

## Researchers

- |   |   |
|---|---|
| ❑ Strongly alkaline ion exchange resins   | Martinez-Castro & Olano, 1980   |
| ❑ Alkaline earth phosphates<br>e.g. disodium phosphate  | Gasparotti, 1981  |
| ❑ Heterogeneous catalysts<br>e.g. MgO, zeolites,<br>sepiolites (hydrated<br>magnesium silicate) | Carobbi <i>et al.</i> , 1985<br>Shukula <i>et al.</i> , 1985<br>Troyano <i>et al.</i> , 1996<br>de la Fuente <i>et al.</i> , 1999<br>Villamiel <i>et al.</i> , 2002 |
| ❑ Egg shell powder  | Montilla <i>et al.</i> , 2005   |

## **Aims & Objectives**

- To study the effectiveness of carbonate-based catalysts
- To evaluate the suitability of Oyster shell powder
- To determine parameters for maximum lactulose yield
- To use lactulose syrup as a prebiotic in dairy products
- To evaluate and compare the probiotic ability of lactulose syrup with commercially available prebiotics

# **Materials & Methods**

# Lactose Source

## 1. Lactose solution

- 4% (w/v) of  $\alpha$ -lactose (95% pure) in distilled water
- Adjusted pH to 6.7 using 0.1 M NaOH

## 2. MCP

- A turbid green-yellowish liquid with 4.5% SNF (~0.5% protein & ~4% lactose) with lot-to-lot variation of 10%
- Collected from the UF unit of a local dairy plant in Victoria, Australia
- Kept by freezing and thawed before isomerisation process.



# Catalysts

## 1. Limestone (pure $\text{CaCO}_3$ )

- A white fine powder

(Analytical-reagent grade, BDH Chemicals, Australia Pty. Ltd.)

## 2. Egg shell powder

- A cream-coloured powder with an average particle size of 117  $\mu\text{m}$

[~94%  $\text{CaCO}_3$ , 1%  $\text{MgCO}_3$ , 1%  $\text{Ca}_3(\text{PO}_4)_2$  and 4% organic matters]

## 3. Oyster shell powder

- An off-white fine powder

(~ 96%  $\text{CaCO}_3$ , ~ 0.696%  $\text{SiO}_2$ , 0.649%  $\text{MgO}$ , 0.984%  $\text{Na}_2\text{O}$  and 0.724%  $\text{SO}_3$ )



# Preparation of Catalyst Powders

## □ Egg shell powders (ESP)

- Removing shell membrane and washing off the residual albumen with tap water
- Drying at 102°C overnight
- Grinding in a micro hammer mill (Glen Mills Inc., USA) (600 *rpm*, 5 min) and sieving through a 120 mesh screen

## □ Oyster shell powder (OSP)

- Cleaning and drying at 102°C overnight
- Breaking into small pieces using mortar, grinding in a hammer mill and sieving through a 120 mesh screen

# Isomerisation Process

*MCP or Lactose solution*

*Catalysts*  
*(12 mg/mL)*

**Mix**

**Heat to 96°C**

**Reflux 96°C/150 min**

**Cool in ice bath**

**Centrifuge**  
**(3600g / 10 min)**

*Supernatant*

# Analyses

1. **pH measurement** (Bench-top pH-meter, model 520A, Orion Research Inc., Boston, USA)
2. **Colour measurement**
  - Chromameter: (model CR-121, Minolta Camera Co., Ltd, Japan) with transmission cuvettes,
  - Spectrophotometer: Shimadzu model UV-1601 (Shimadzu Cooperation, Australia)

### 3. HPLC analyses

#### □ HPLC System

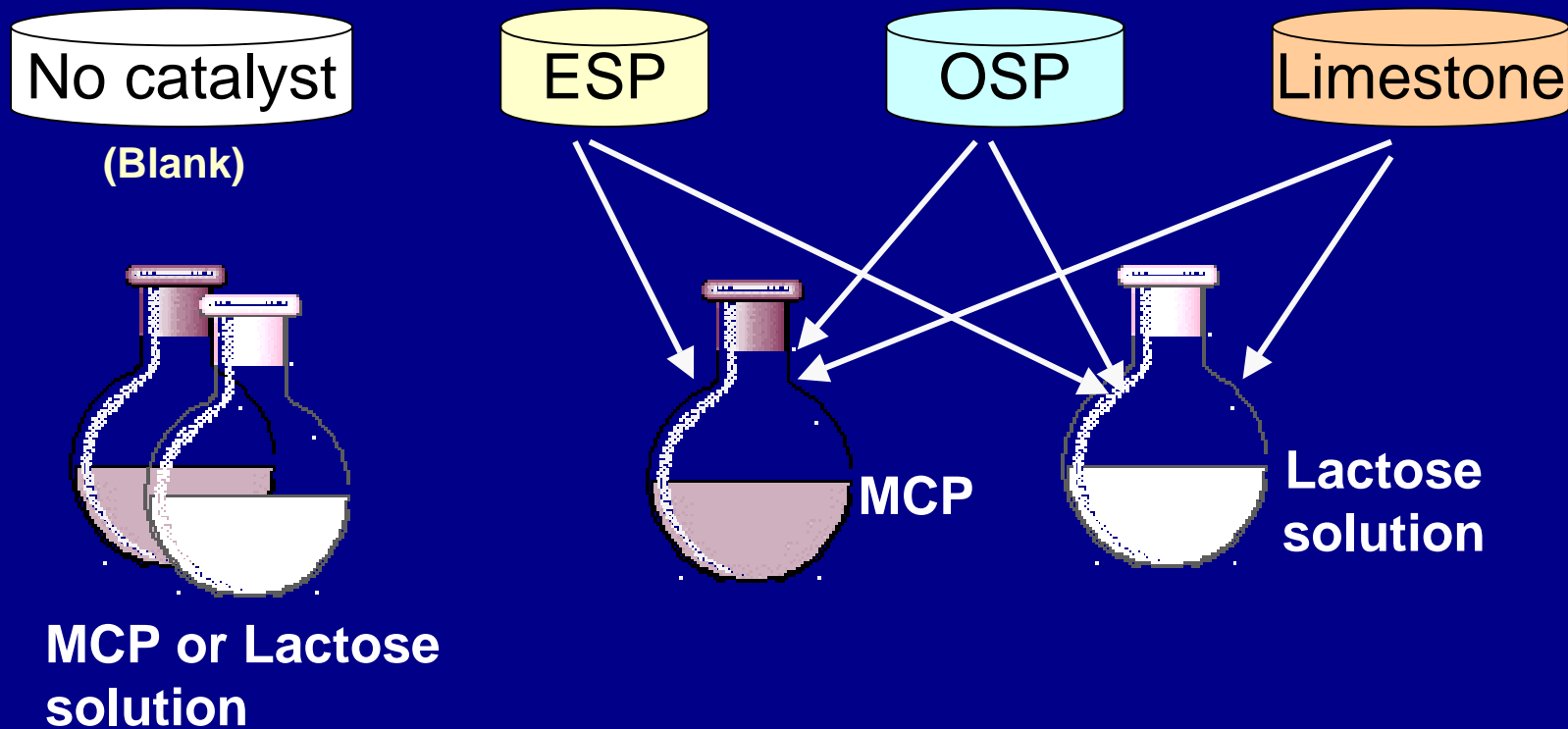
- Waters Model 6000A pump module (Waters associates Inc., Milford, MA, USA)
- A 20-ml injection loop (Rheodyne®, Cotati, CA, USA)
- A 3.9×300 mm stainless steel carbohydrate analysis column (Waters Co., USA)
- A Waters Model 41 refractive index detector (RID)

#### □ HPLC Conditions

- Mobile phase: degassed and filtered 80:20 (v/v) acetonitrile-water
- Flow rate: 0.8 mL/min
- Column temperature: ambient

# Experimental Design

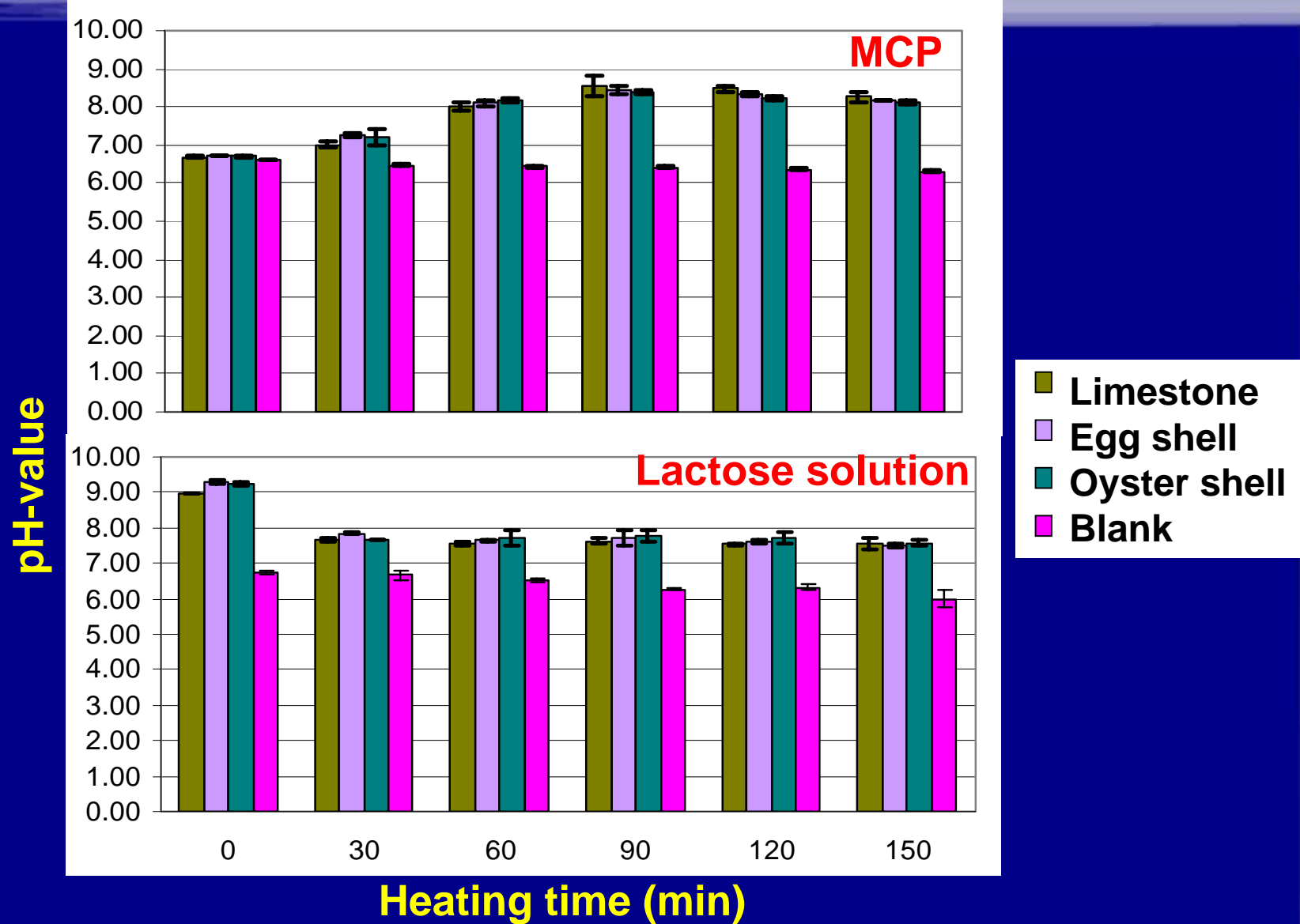
- ❑ Comparing the lactose isomerisation ability of different catalysts
- ❑ To establish the optimum conditions for obtaining maximum lactulose yield



# Results & Discussion

## Effect of pH on lactulose formation

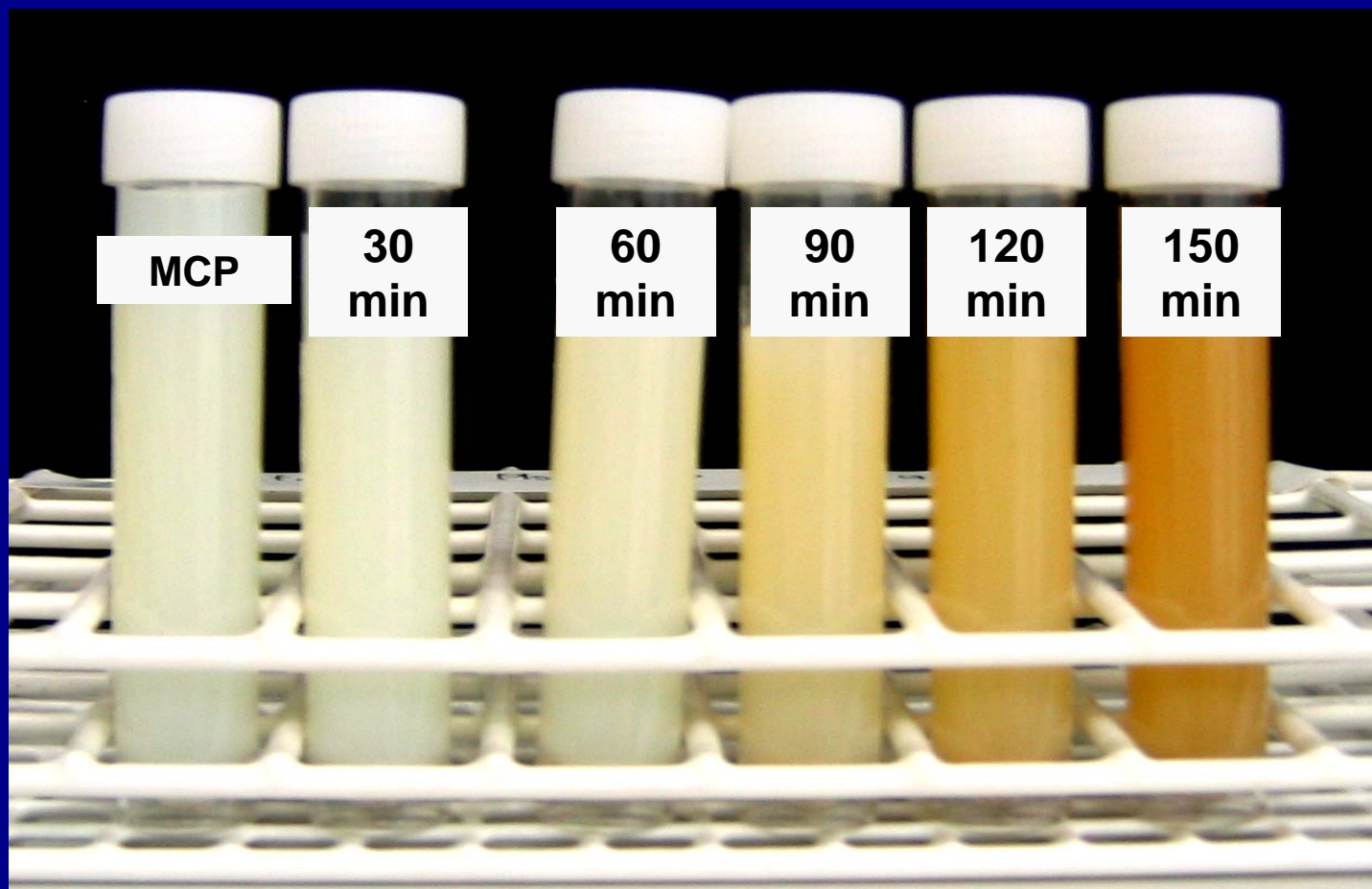
- pH was measured every 30 min during heating
- At the initial pH (6.6-6.7) no lactulose formed in MCP or lactose solution
- Upon the addition of catalysts:
  - Significant pH rise (>9) in lactose solution
    - → lactulose was formed within 30 min of heating
  - Only slight pH rise (~6.8) in MCP
    - → no lactulose form within 30 min



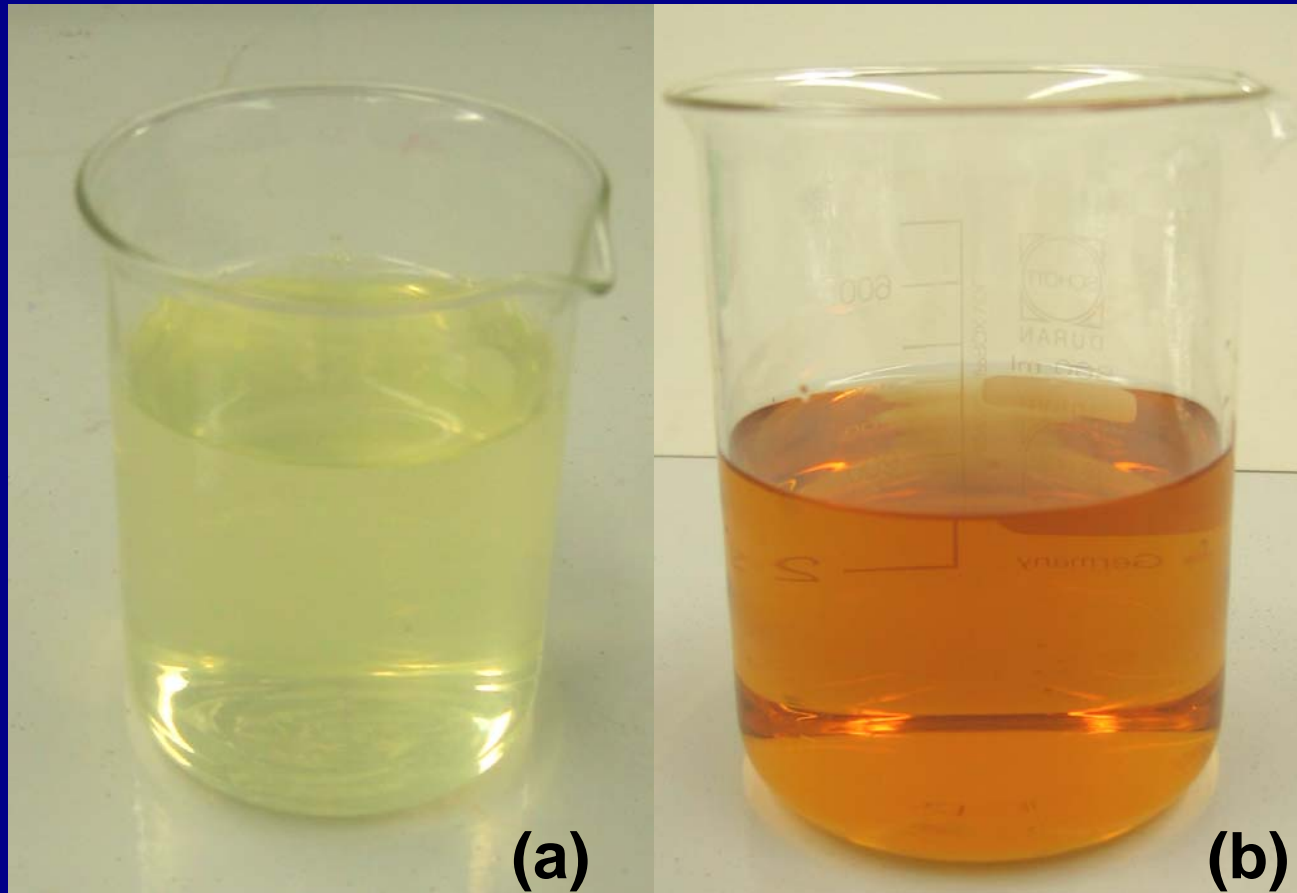
**Figure 3.** Changes in pH of MCP and lactose solution during isomerisation using different catalysts

- ❑ Buffering effect of proteins in MCP may be the cause of pH stability in MCP
- ❑ The subsequent rise in pH of MCP to >8 after 60 min may be due to proteins denaturation and loss of their buffering capacity
- ❑ Prolonged heating caused lactulose degradation and depressed the pH in both solutions, indicating the formation of organic acids i.e. isosaccharinic and formic acids (Berg & van Boekel, 1994)
- ❑ pH was used as the end point indicator

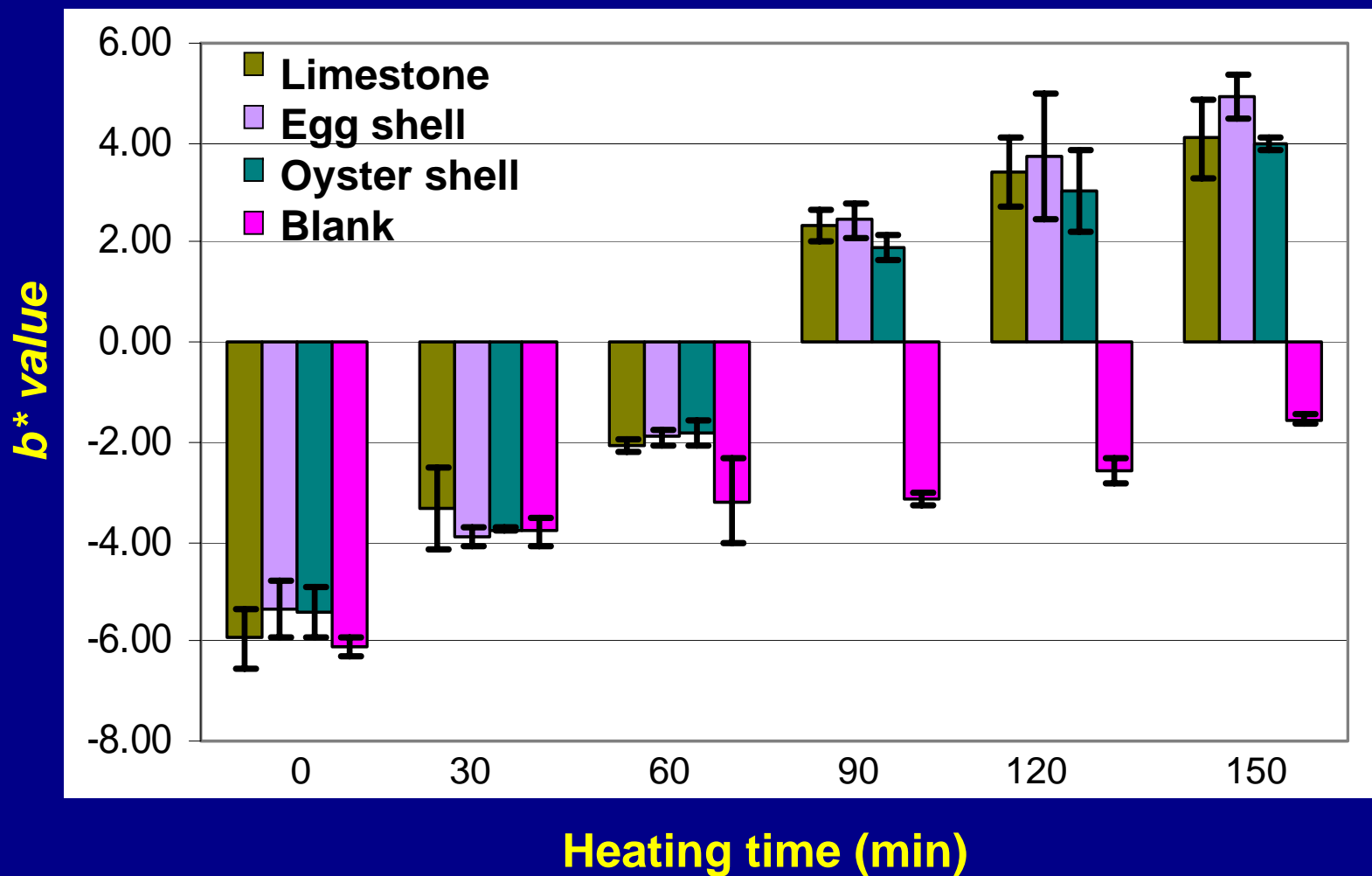
# Colour Change During Isomerisation



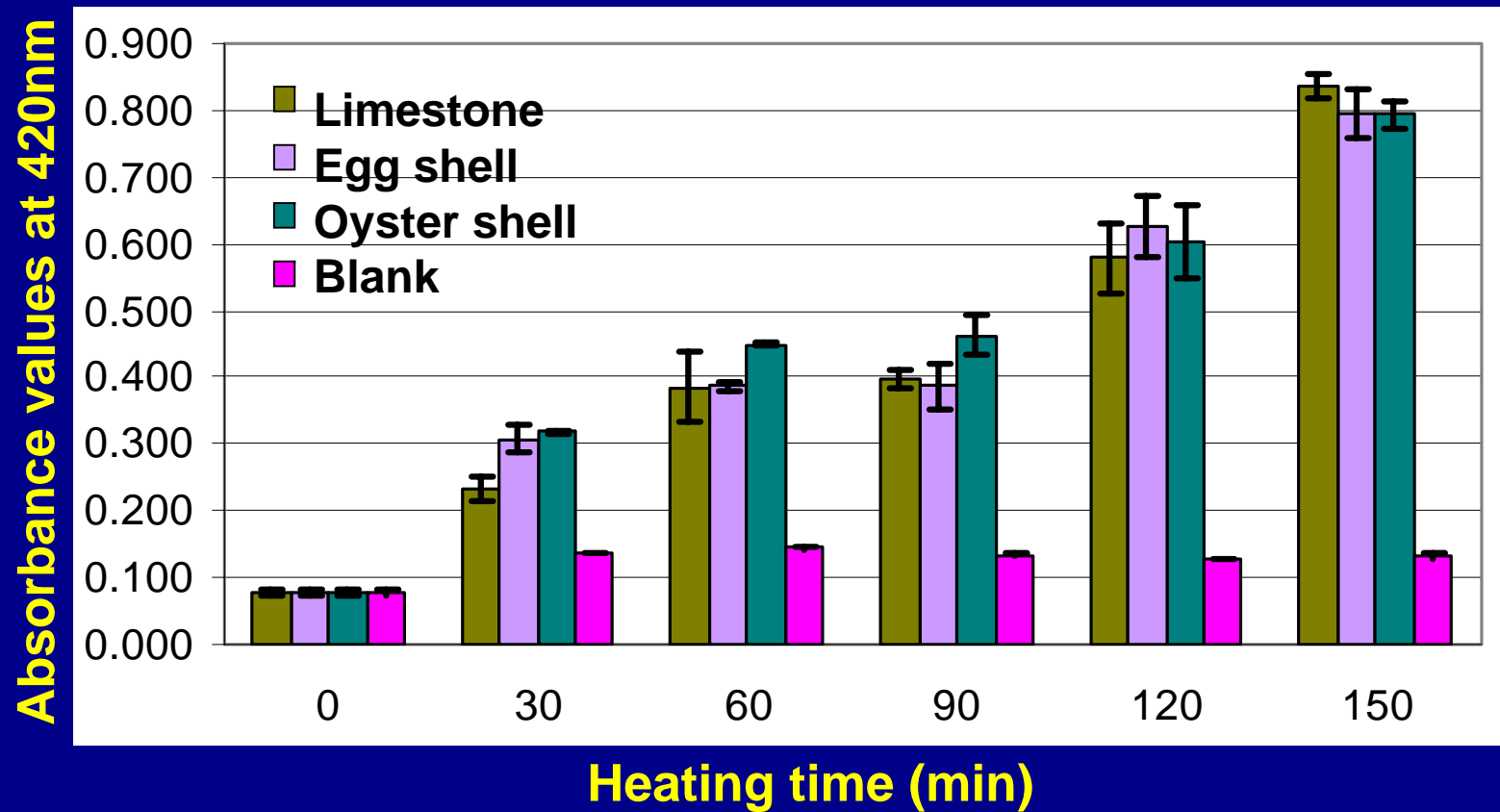
**Figure 4.** MCP Colour change at 96°C with 12 mg/mL catalyst



**Figure 5.** De-proteinated MCP (a) before and (b) after isomerisation at 96°C for 120 min with 12 mg/mL egg shell powder

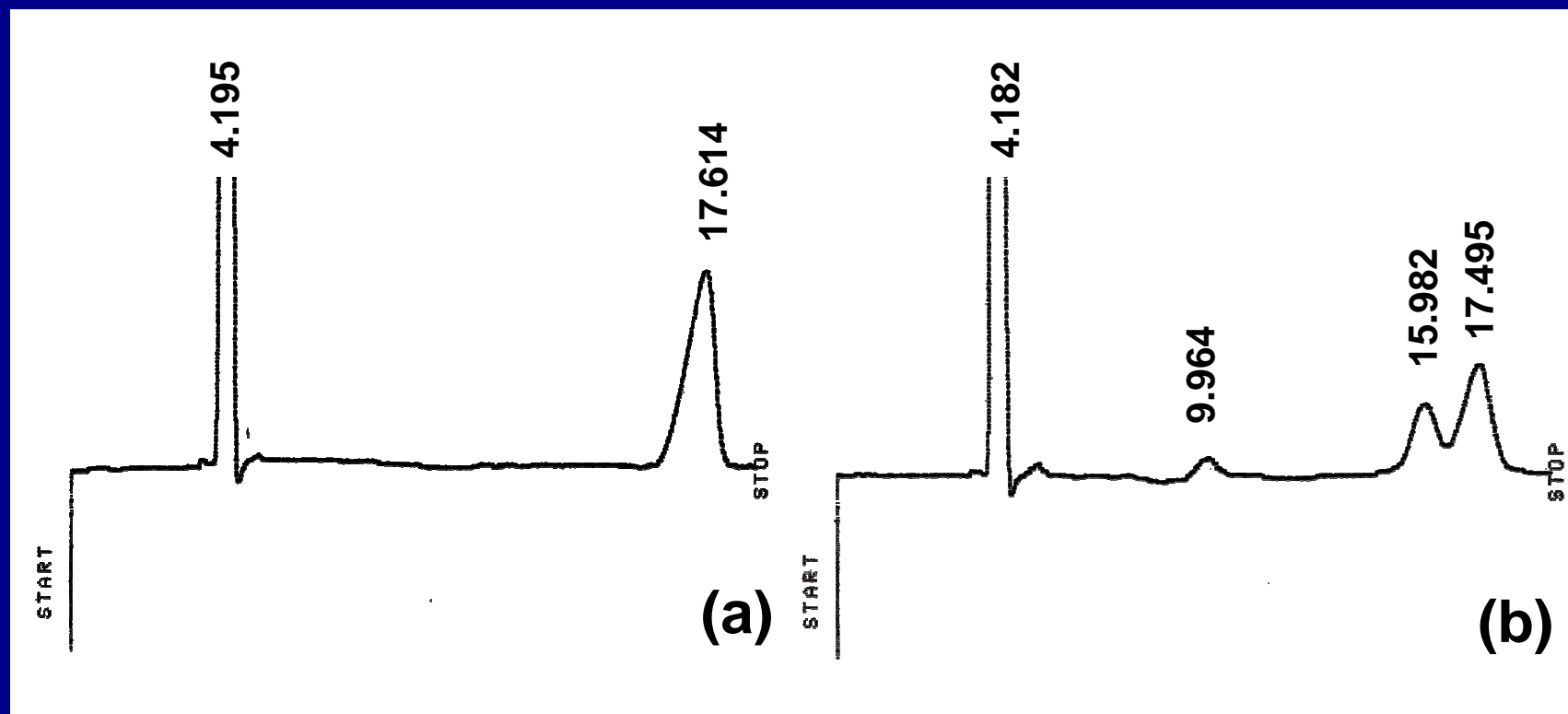


**Figure 6.** Increase of  $b^*$  values during isomerisation of MCP using different catalysts



**Figure 7.** Increase of absorbance values during isomerisation of lactose solution using different catalysts

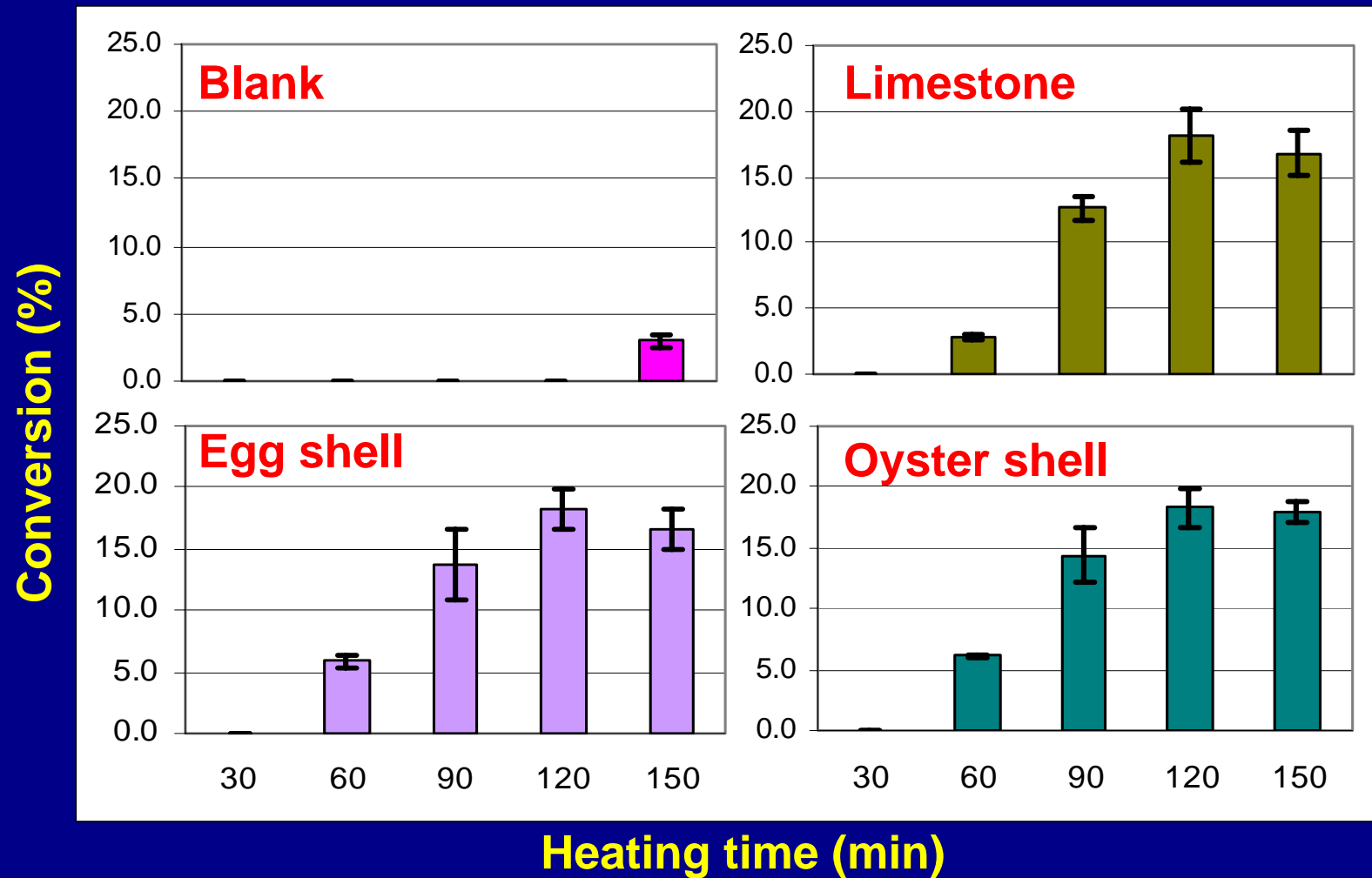
## HPLC determination of lactulose and lactose content



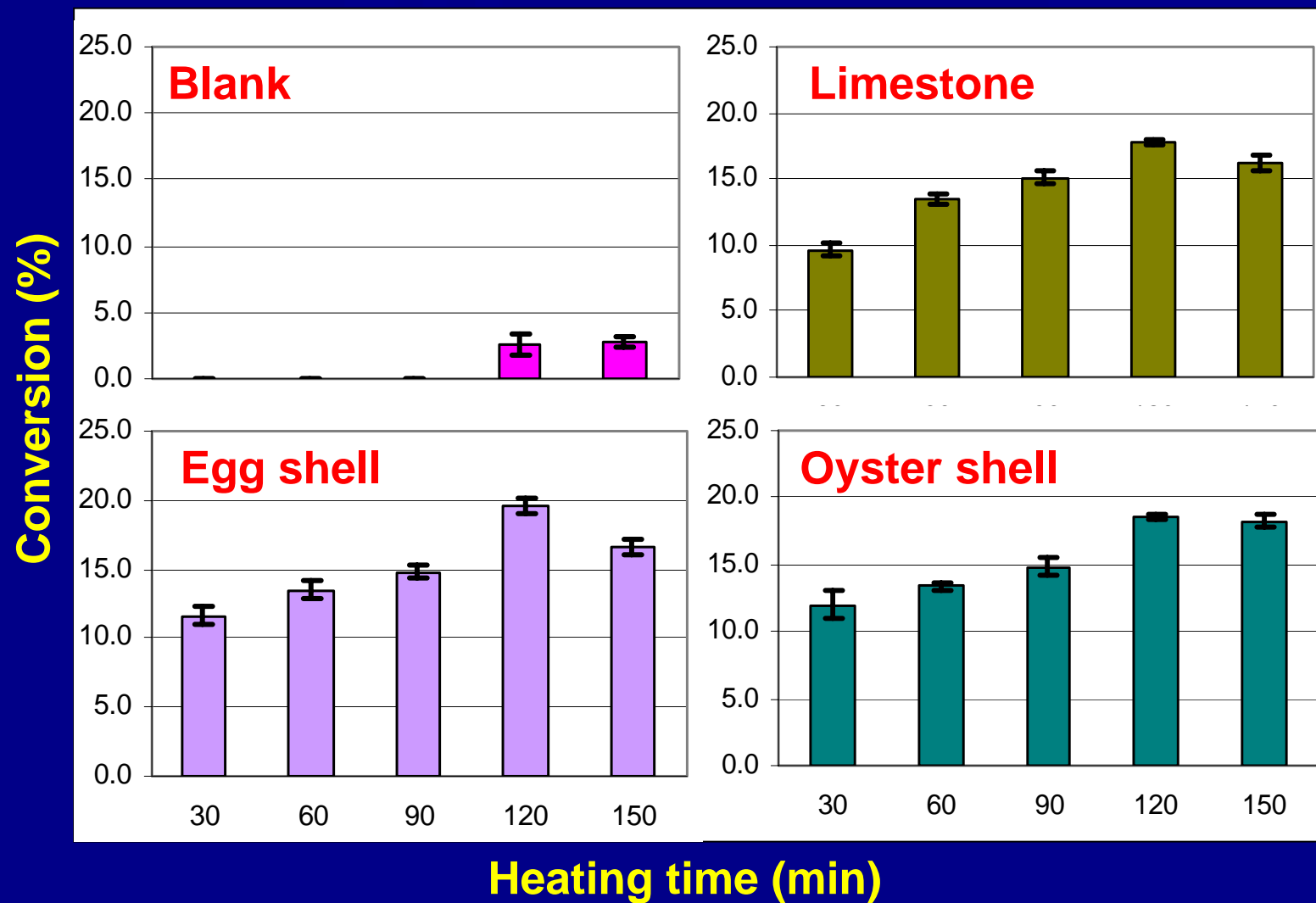
**Figure 8.** HPLC-RID chromatogram of MCP (a) before and (b) after isomerisation at 96°C for 120 min

Solvent (4.20 & 4.18), lactose (17.61 & 17.50), galactose (9.96) and lactulose (15.98)

- ❑ Lactose content dropped in favour of lactulose formation
- ❑ The presence of galactose peak (Fig.8b) indicates the decomposition of lactose or lactulose
- ❑ In the absence of catalyst the conversion level was very low and only 3% lactulose formed after 120-150 min isomerisation (Fig. 9 & 10)
- ❑ In the presence of catalyst, significantly higher level of conversion was observed and in the earlier stages of heating



**Figure 9.** Effects of catalyst type on the conversion of lactose to lactulose in MCP



**Figure 10.** Effects of catalyst type on the conversion of lactose to lactulose in lactose solution

- ❑ In the earlier stages of heating, lower level of lactulose formed in MCP than in lactose solution due to:
  - The buffering effect of proteins and peptides
  - The reduction of available lactose and/or lactulose through the formation of lactosyl- and/or lactulosyl-amino compounds
- ❑ Changing catalyst type had only a small effect on the conversion rate of lactulose
  - Limestone in MCP achieved:
    - 3% less conversion than shell powders after 60 min,
    - 2% less in lactose solution after 30 min
  - The effectiveness of all catalysts was similar between 90 and 150 min in both solutions.

Degradation of lactose (%)

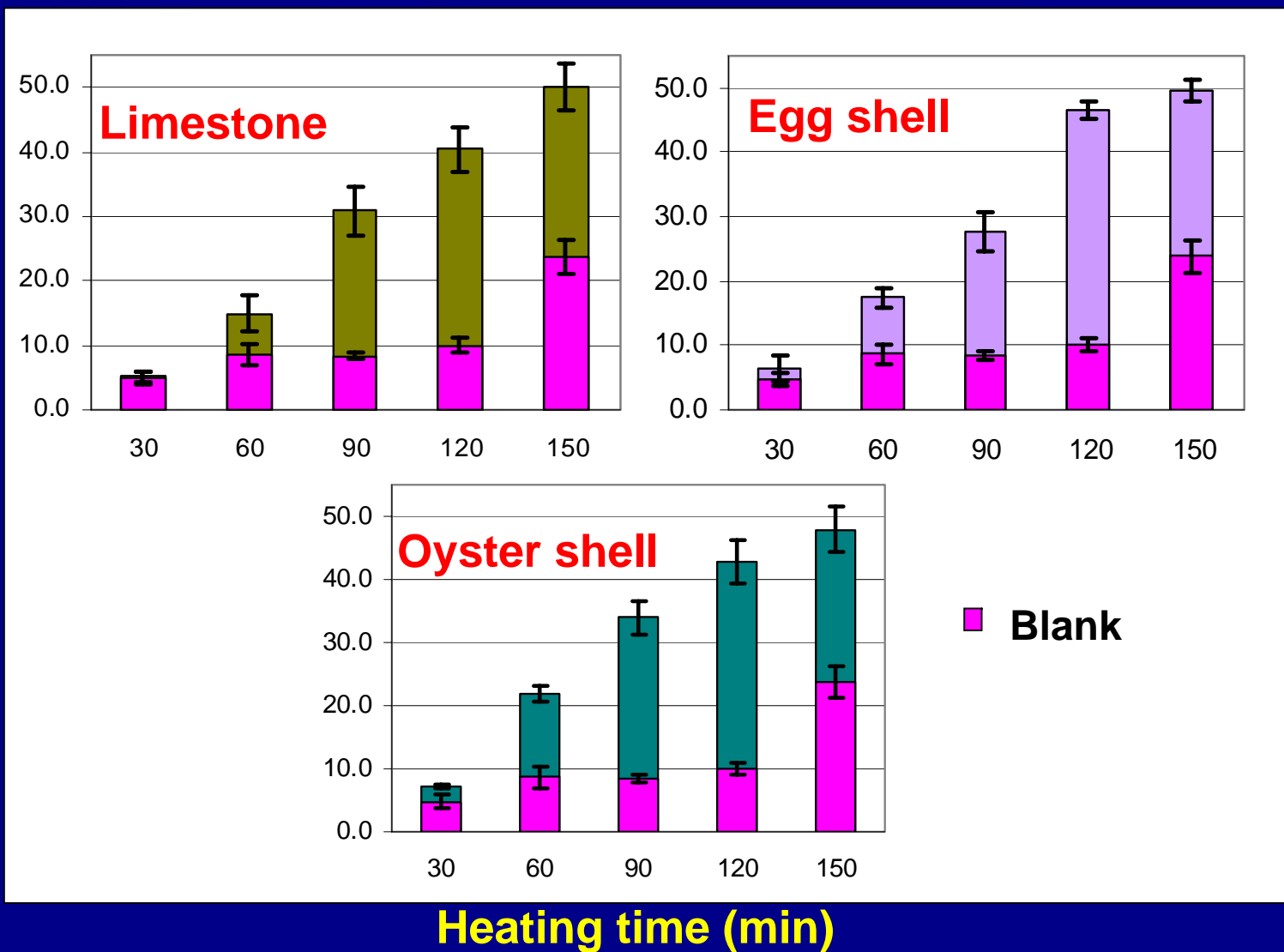


Figure 11. Effects of catalyst type on the degradation of lactose in MCP

Degradation of lactose (%)

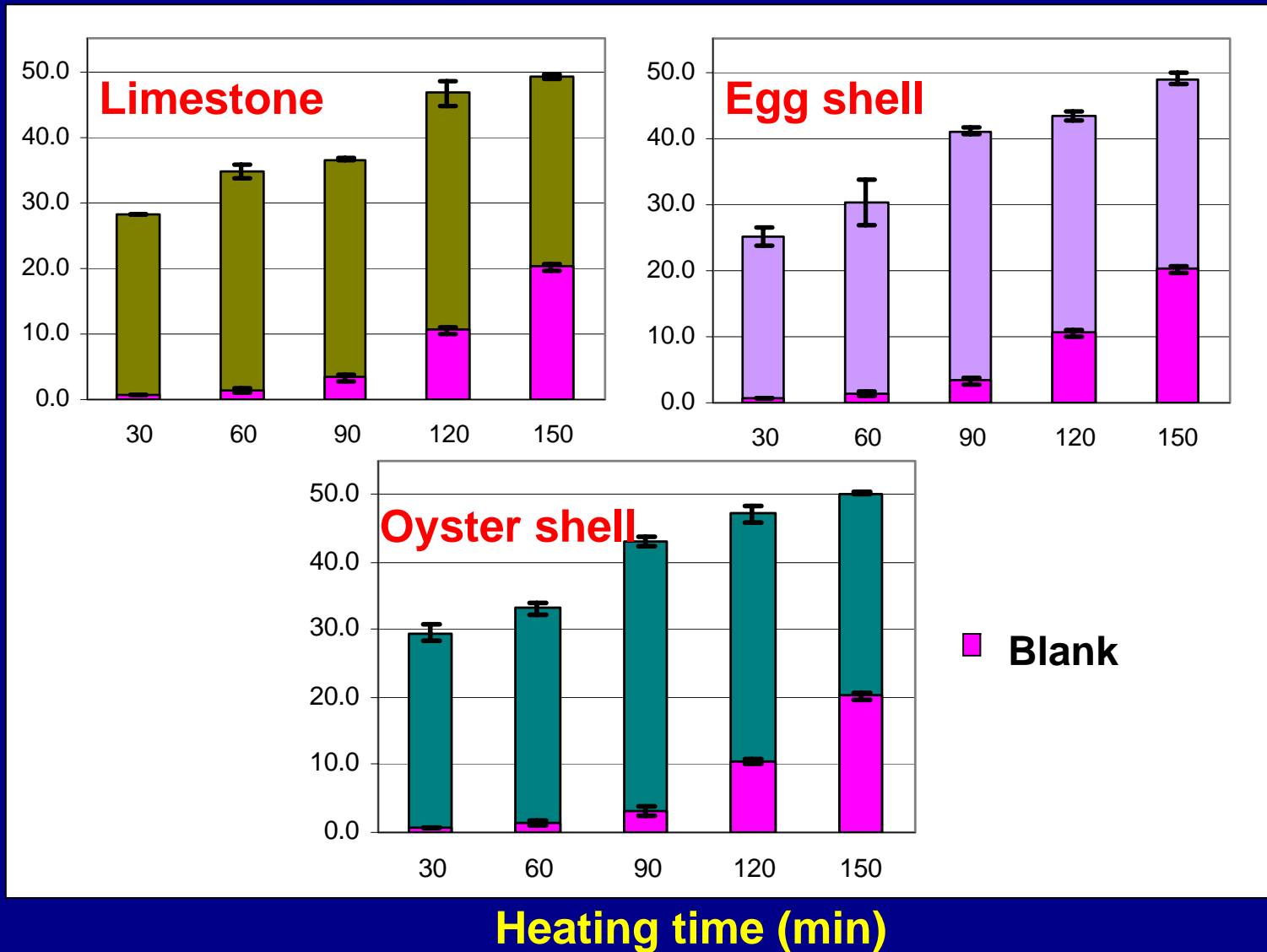
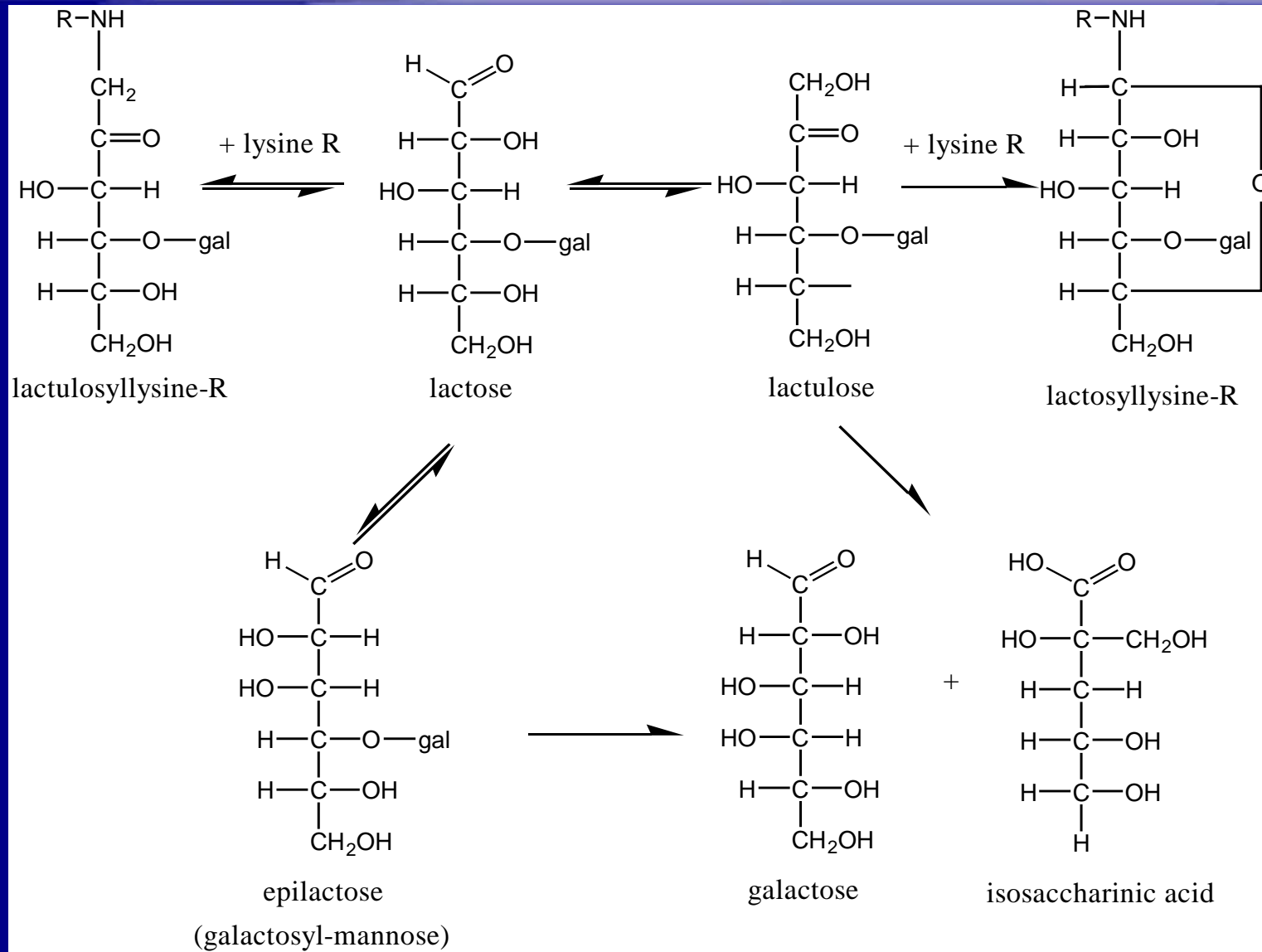
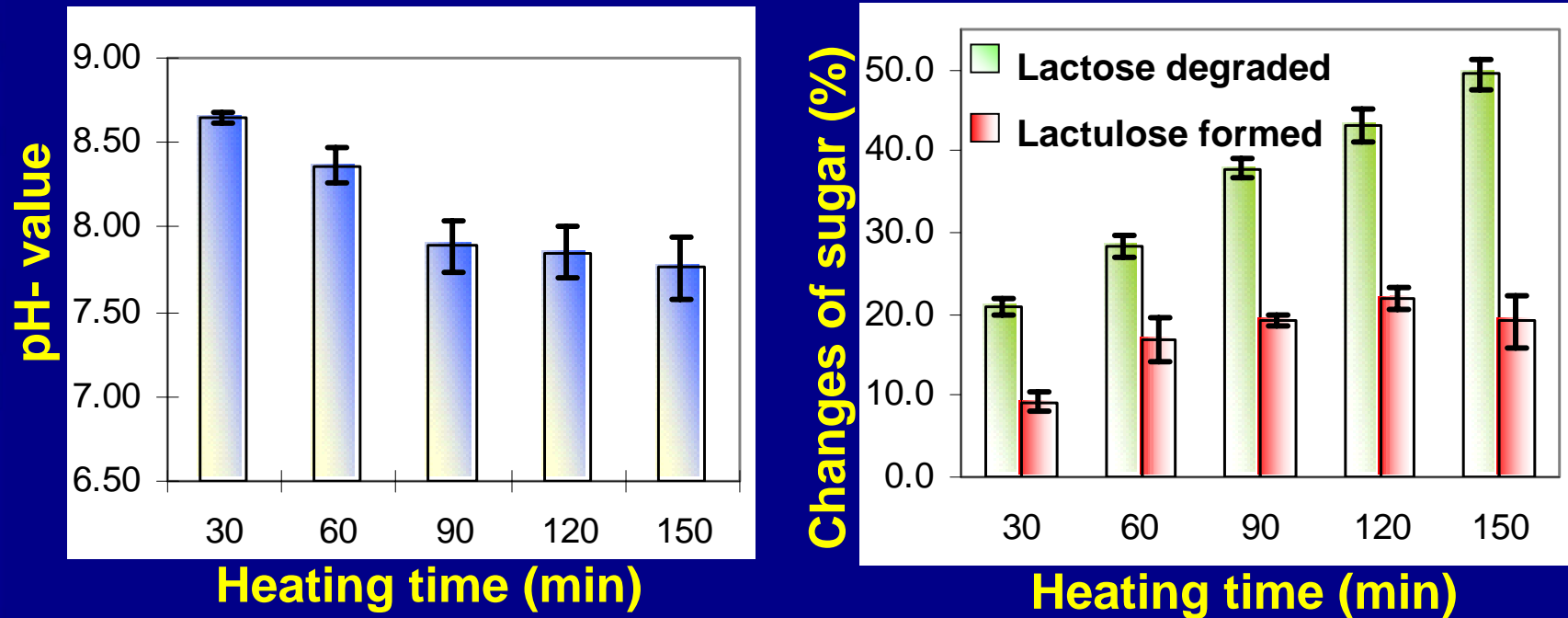


Figure 12. Effects of different catalysts on the degradation of lactose in lactose solution



**Figure 13. Scheme for isomerisation and degradation of lactose**  
 (Adapted from Berg & van Boekel, 1994)

# Removal of Protein



**Figure 14.** Effect of de-proteination of MCP on lactose isomerisation

## Future works

- ❑ Concentration of the resulting syrup to 40°B
- ❑ Removal of residual minerals from concentrated syrup
- ❑ Investigation of the bifidogenic property of lactulose syrup in probiotic milk model

## Conclusions:

- ❑ MCP can be used as convertible source of lactose
- ❑ Residual proteins in MCP may adversely affect the yield of lactulose
- ❑ Oyster shell powder gave comparable yield of lactulose with egg shell powder and limestone
- ❑ With the optimum conditions of 120 min at 96°C, a maximum yield of 18-21% lactulose was achievable to the net cost of <37% of original lactose degradation
- ❑ The resulting syrup can be directly added to food formulations as a food-grade prebiotic.

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# спасибо

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